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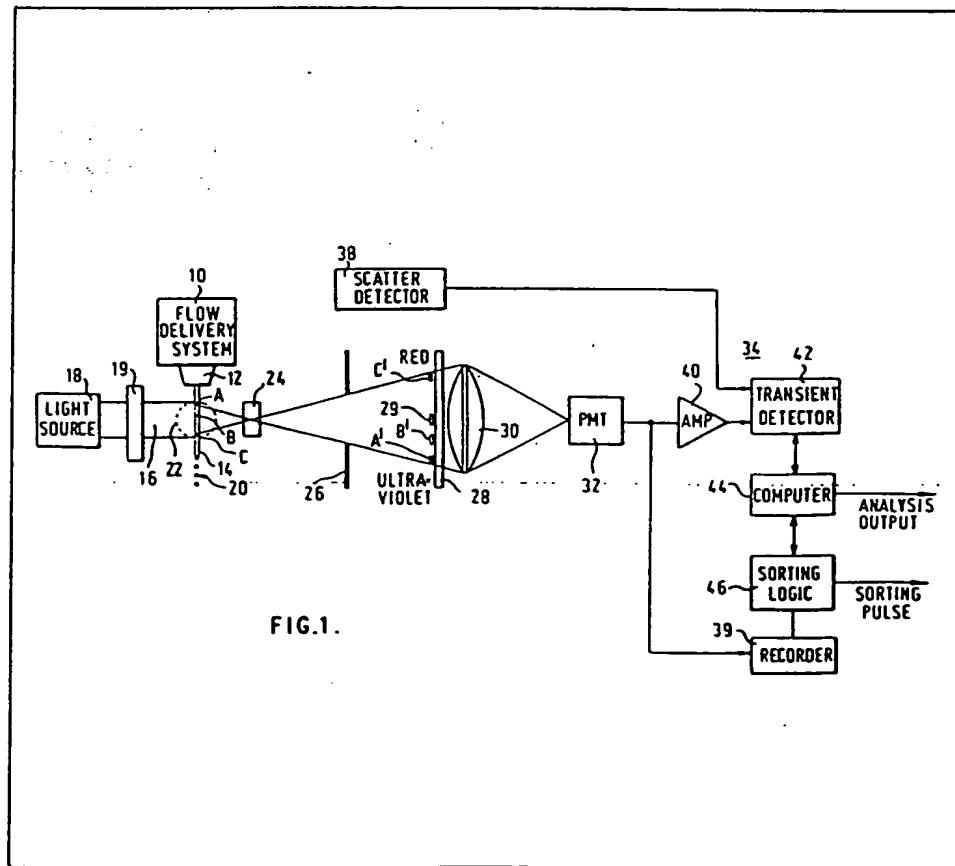
# UK Patent Application (19) GB (11) 2 032 097 A

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## (54) Apparatus and method of producing the fluorescent emission spectrum of particles

(57) A flow microfluorometer has a delivery system (10) which delivers a stream (14) containing particles or cells. The stream (14) traverses a light beam (16), the image of each particle being projected onto a wedge filter (28) or similar dispersion device. The wedge filter is such that as the image of each particle moves across its

surface from (A' to C') it sequentially transmits light of increasingly longer (or shorter) wavelength. The radiation transmitted from the wedge filter (28) is sequentially focused onto a photomultiplier tube (32) which provides an electrical signal which is a function of the wavelength of the output of the filter. The amplified output of the photomultiplier tube is fed to signal processing circuitry to analyse the output waveforming and thereby provide an analysis of the cell population under investigation.



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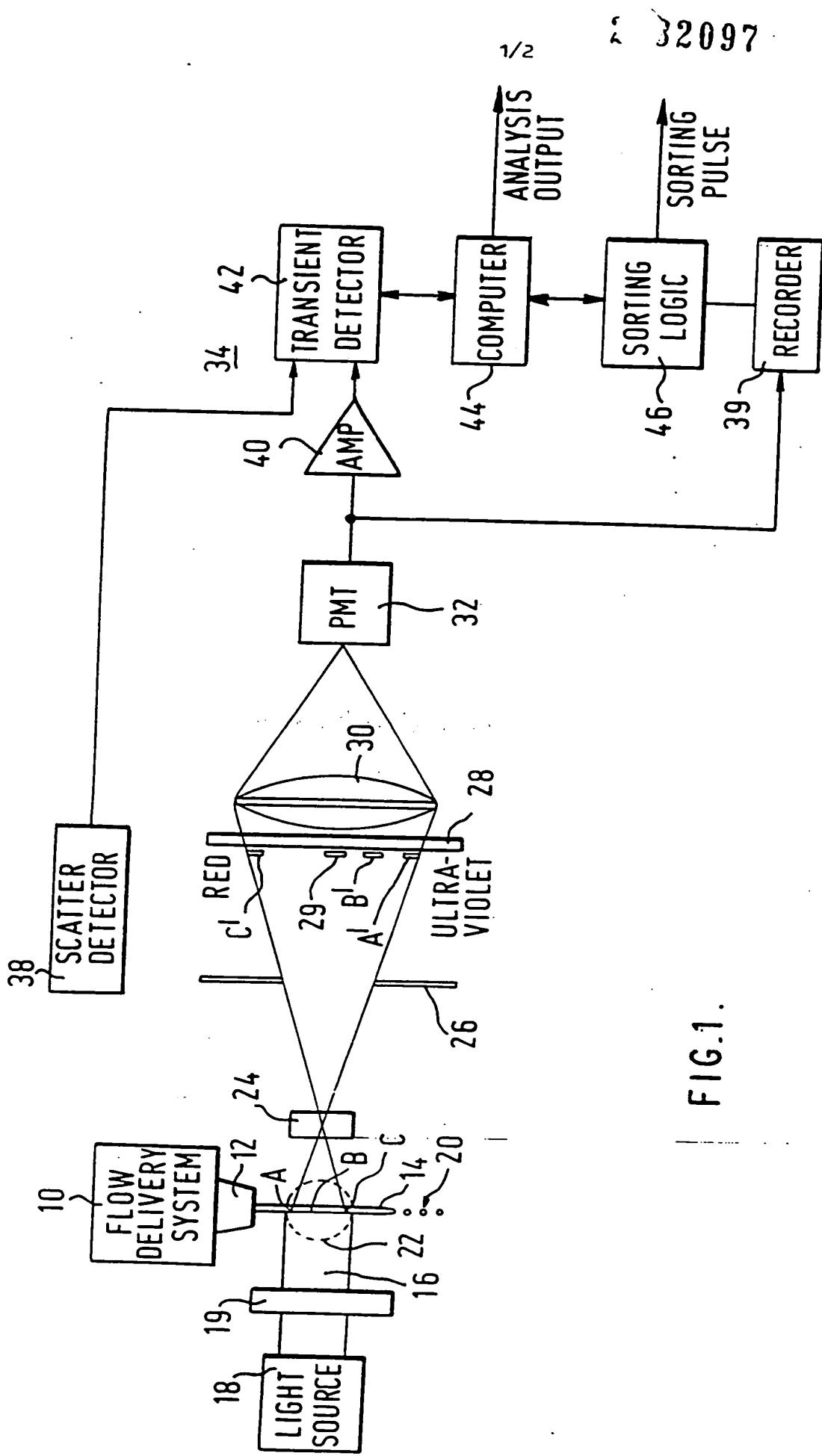


FIG.1.

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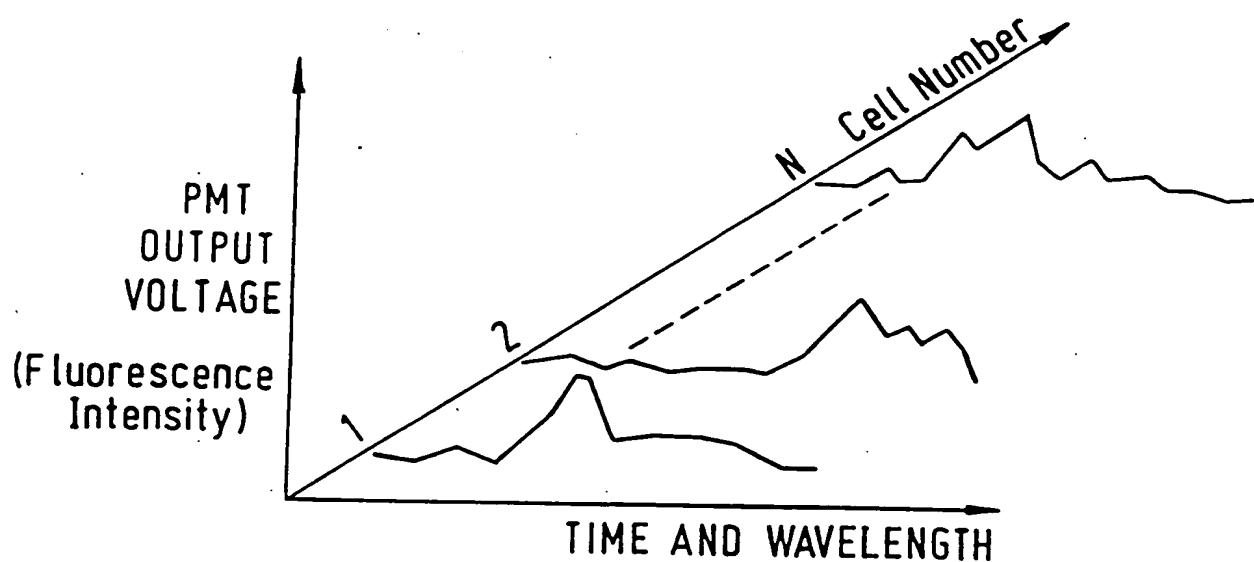


FIG. 2.

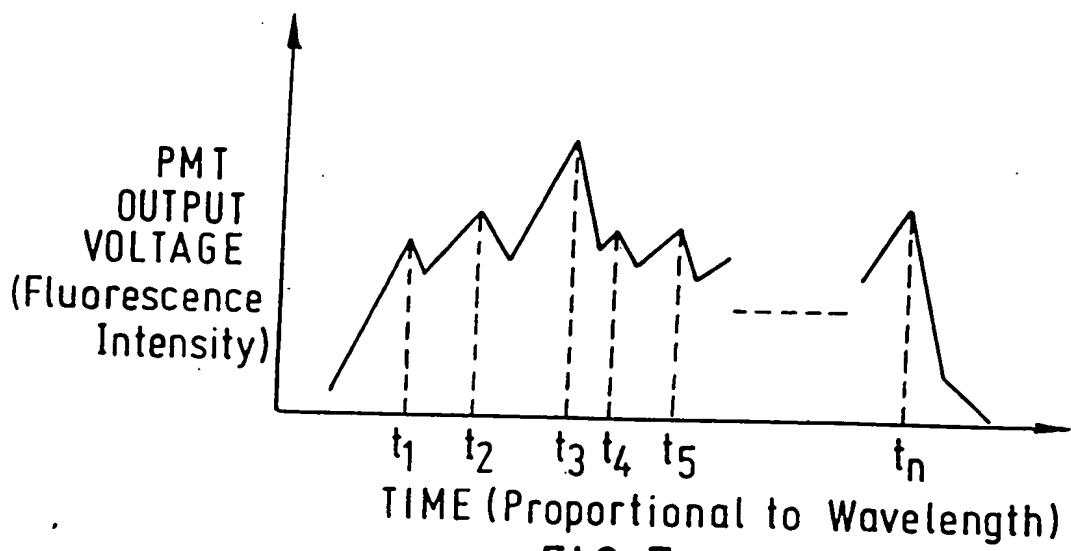


FIG. 3.

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## SPECIFICATION

## Apparatus and method of producing the fluorescent emission spectrum of particles

This invention relates to apparatus for and a method of producing the fluorescent emission spectrum of particles.

As is known, the fluorescence spectrum of stained or unstained particles such as human cells can yield valuable information as to the nature of the cell and/or the condition of the cells. For example, the emission spectra of various stained particles within the blood are different. Thus, a count of the different types of white blood cells can be obtained since the emission spectra of these cells are different. Further, it is known the emission spectra of certain stained cancer cells are different from those of normal cells in certain tissues of the body. Again, the differences in emission spectra can be utilized to detect the abnormal presence of pathogenic cells.

It is known to accumulate spectra on individual cells by static single cell analysis or upon a stationary solution containing a number of cells, such as disclosed in U.S. Patents 3 470 373, 3 497 690, 3 918 812, and 3 973 129. Further, it is known on a static basis to project a real image of a cell to a predetermined point, there being disposed at a point a rotating or oscillating wedge filter or grating whereby a continuous fluorescence emission spectrum of the static cell can be obtained. However, the accumulation of spectrum information is quite slow due to the static nature of the procedure. The use of a wedge filter in a spectrophotometer is also disclosed in U.S. Patent 3 885 879.

Data can be more rapidly accumulated by dynamic single cell analysis in flow microfluorometers where cells are permitted to pass through an excitation light beam to effect fluorescence thereof where, for example, 500—5,000 cells per second can flow through the beam. Such flow microfluorometers are disclosed, for example, in U.S. Patents 3 586 859, 3 864 571, and 3 916 197. In some of the latter microfluorometers, at least two filters are employed, each of which passes a different wavelength of the fluorescence emission spectrum of the activated particles. The filter outputs are then respectively focused at at least two different photomultiplier tubes. The outputs of the photomultiplier tubes are then combined for appropriate signal processing.

The resolution of the foregoing flow delivery systems is that only two wavelength portions or lines are detected out of the entire fluorescence emission spectrum of the particles. It is possible to increase this resolution by employing more filters and accordingly more photomultiplier tubes, there being, as indicated above, one photomultiplier tube for each filter. Of course, the resolution can be increased in this manner only to a certain point at which either space limitations or cost limitations occur. Not only is each photomultiplier tube expensive but also there are many

65 applications where space is at a premium.

A further limitation of the above described microfluorometers is that the light supplied to the filters is divided between the filters. Hence, the light available at each filter output for detection is somewhat reduced thus rendering the system somewhat inefficient.

It is accordingly an object of this invention to provide improved apparatus for and a method of producing the fluorescent emission spectrum of particles.

According to one aspect of this invention there is provided apparatus for producing the fluorescence emission spectrum of particles comprising means for passing said particles 80 through an excitation light beam to effect fluorescence thereof; and detecting means for detecting predetermined wavelengths of the said fluorescence emission spectrum of each particle, each detected wavelength being a function of the 85 position of said particle with respect to said detecting means as it passes through the excitation light beam.

According to another aspect of this invention, there is provided a method for producing the 90 fluorescence emission spectrum of particles comprising the steps of passing said particles through an excitation light beam to effect fluorescence thereof; and detecting with a detecting means predetermined wavelengths of the 95 said fluorescence emission spectrum of each particle, each detected wavelength being a function of the position of said particle with respect to said detecting means as it passes through said excitation light beam.

100 This invention can provide a flow microfluorometer apparatus and method whereby a continuous or line fluorescence emission spectrum of a particle can be rapidly obtained.

105 A microfluorometer and method according to this invention may employ a single light detector.

A microfluorometer and method according to the invention can provide the fluorescence emission spectrum of the particle rapidly in a sequential manner.

110 A microfluorometer and method according to this invention can process human cells.

The light may be efficiently utilized at the light detector.

An embodiment of this invention will now be described, by way of example, with reference to the accompanying drawings of which:

Figure 1 is a schematic diagram of flow microfluorometer in accordance with the invention;

120 Figure 2 is an illustrative X—Y oscilloscope display of the spectrum of N cells in accordance with the invention; and

125 Figure 3 is a graph which illustrates the temporal variation of the spectrum of a cell and how different wavelengths can be obtained at different points in time.

Referring to Figure 1, there is shown a flow delivery system 10 having a nozzle 12 which delivers a particle containing stream 14. The

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particles may either be endogenous whereby they fluoresce upon proper excitation thereof or they may be tagged with appropriate substances to effect fluorescence thereof. Further, the particles may 5 comprise cellular matter of either the animal or plant kingdoms. Although there is no intent to be limited to a particular flow rate, typically 500—5,000 cells per second are sequentially delivered from nozzle 12 with a cell separation of 10 0.2—2 cm., the particles transversing a beam 16 emitted from a light source 18 where the cells pass through the beam single file. As indicated at 20, the particle containing fluid tends to break up into drops after passage through the beam. Flow 15 delivery system 10 may correspond exactly to that incorporated in the Fluorescence Activated Cell Sorter (FACS II) manufactured by Becton, Dickinson and Company, Rutherford, New Jersey.

The cross-sectional area of the beam is 20 indicated at 22 in dotted lines, the diameter thereof being typically approximately 1 millimeter. The source 18 may correspond to the laser source used in the above-mentioned Becton, Dickinson FACS II system. If so, the light source is 25 orthogonal to the said detection system at the stream. It should be noted that a 50 micron diameter laser beam is employed in the FACS II system whereas, as mentioned above, the beam width employed in the present embodiment may 30 typically be 1 millimeter. This larger diameter beam can be obtained by simply defocusing the smaller FACS II beam. Of course, laser sources are also known which are capable of generating a 1 millimeter beam. In any event, there is no 35 invention to be limited to a particular width beam nor is there any intent to be limited to a particular type beam or source in that, for example, an ultraviolet source may also be used as source 18.

Further, cylindrical lens or other appropriate 40 one dimensional converging means may be disposed as shown at 19 to converge the beam from source 18 into a line coaxial with the path through the cells travel, the line extending between A and C in Figure 1. By so converging the 45 beam, the intensity thereof is limited to the path travelled by the cells to thereby optimize the utilization thereof.

A particular cell is shown in three different 50 positions A, B and C as it passes through the beam. The cell is, of course, fluorescence activated as it passes through the beam. A lens system 24 and an iris 26 project the image of each cell onto a wedge filter 28, the image of the cell at position A being projected to position A' on filter 28, the 55 image at position B being projected to B' and C to C'. An obscuration bar 29 acts to block the excitation at its transmission region on the wedge filter. In addition to or instead of, a barrier filter can be used to block the excitation. As indicated in 60 Figure 1, the lower end of filter 28 passes radiations at the ultra-violet end of the spectrum, the upper end passes red wavelengths while position B' may correspond to blue wavelengths. Hence, although the entire fluorescence emission 65 spectrum is projected onto A' when the cell is at

A, only a short wavelength portion of the spectrum (ultraviolet, for example) is passed by the filter. At B', a longer wavelength portion is passed (blue, for example) while at C' the longest wavelength portion of the spectrum is passed (red, for example). The radiations transmitted from filter 28 are sequentially focused by a collecting lens 30 onto a photomultiplier tube 32. That is, when the cell 70 is at A', the shorter wavelength portion of the fluorescence emission spectrum is focused onto the photomultiplier tube. As the cell passes through the beam 16, successively longer wavelength portions of the emission spectrum will be focused at tube 32 until the cell image reaches C' at which time the longest wavelength of interest will be directed to the tube. Hence, the output signal from photomultiplier tube 32 which is applied to a signal processor 34 and a recorder 75 39 is a time varying signal, the time axis thereof being proportional to wavelength and the voltage or current output being proportional to the emission intensity at a particular wavelength. It can thus be seen from the foregoing that there is provided a 80 unique method for scanning the entire fluorescence emission spectrum of particles such as cells, this being effected by projecting the moving image of the particle as it passes through an excitation beam onto a wedge filter which 85 thereby sequentially transmits increasingly longer (shorter) wavelength portions of the spectrum to a detection device including lens 30 and tube 32. In lieu of wedge filter 28, other optical dispersion means may be used such as a prism or grating 90 where suitable geometry would be employed and the principle would be the same. Also, in lieu of lens 30 and tube 32, a linear light sensitive, diode array may be placed in the position of lens 30 adjacent filter 28 whereby the lowest diode would 95 be responsive to the ultraviolet portion (for example) of the spectrum and the highest, the red portion (for example) thereof. In this case, the outputs from the diodes would still occur sequentially; however, they would be in parallel 100 and suitable for processing by appropriate circuitry. It should also be noted that although the wedge filter 28 has been shown with the red end thereof at the top and the ultraviolet end at the bottom, these ends could be reversed so that red 105 occurs at the bottom and ultraviolet at the top. The spectrum as generated above is the so-called uncorrected spectrum and may be corrected by 110 correcting for the system transmission function either electronically or by the use of spatial filters 115 as is known in this art.

The output signal from photomultiplier tube 32 is applied to a signal processor generally indicated at 34 the operation of which may be initiated by the output of a scatter detector 38. Scatter detector 38 is 120 responsive to light scattered from each cell when it reaches point A. That is, prior to the cell reaching point A, it does not fluoresce. Upon reaching point A, the fluorescence is activated by the laser beam and, in addition to the rays 125 projected through lens system 24 and iris 26,

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there are rays which impinge upon detector 38. Detector 38 includes a photodetecting element and may correspond exactly to the scatter detector used in the above-mentioned FACS II system. Photomultiplier tube 32 may also correspond to the tube used in the FACS II system as may lens system 24 and iris 26. Scatter detector 38 thus signals to processor 34 the presence of a particle in beam 16. The output from photomultiplier tube 32 may also be applied to a recorder or display 39 such as an oscilloscope where the fluorescence spectrum may be visually inspected and photographed if desired. For example, X-Y oscilloscope may be employed to isometrically display each pulse (or cell) as shown in Figure 2.

Signal processor 34 effects the extraction of more information. Thus, it permits analysis of the entire output waveform from tube 32 and computer analysis of the entire cell population. Thus, the output waveform is applied to a transient waveform detector 42 via an amplifier 40 and then fed to a computer 44. Detector 42 and computer 44 are commercially available and the storing logic may be the same as that used in the FACS II system. With appropriate *a priori* knowledge, it is possible to quantitate those compounds which fluoresce in the particle. Wavelength variation in spectra can be obtained at different points in time as indicated in Figure 3. Each  $t_i$  corresponds to a particular fluorescence wavelength, and the value of  $n$  is determined by the overall resolution of the chemical (tagging) optical and electronics system. An appropriate data reduction scheme may be used to obtain descriptors of the spectrum which then may be used as a means of identifying similar cell types. It is possible to physically isolate cell types according to the values of fluorescence at a particular wavelength or by use of the descriptors. Since the grouping of the cells is dependent upon its identification, it suffices only to find the appropriate means of analysis and to sort in accordance with sorting logic 46 on the basis of this analysis, as is possible on the cell sorter-type of instrument. Care is required to consider uniqueness for the descriptors and the time available to perform the sorting operation. It is apparent that many schemes exist to analyze and sort cells on the basis of their fluorescence spectra as measured in the present invention. For example, one can measure the fluorescence depolarization spectrum of single particles by appropriately splitting the emission beam with polarizing filters and using two of the units of the present invention. Both the parallel and perpendicular fluorescence quantities are available for calculation using the approach of processor 34 of Figure 1. Many other examples can be implemented by using alternate schemes with the described embodiment of the present invention and appropriated electronics.

#### CLAIMS

##### 1. Apparatus for producing the fluorescence

65 emission spectrum of particles comprising means for passing said particles through an excitation light beam to effect fluorescence thereof; and detecting means for detecting predetermined wavelengths of the said fluorescence emission

70 spectrum of each particle, each detected wavelength being a function of the position of said particle with respect to said detecting means as it passes through the excitation light beam.

2. Apparatus as claimed in claim 1 wherein said detecting means includes optical dispersion means having at least a first surface; means for projecting and image of each said particle onto said optical dispersion means so that the particle image moves across said first surface as the particle passes through said excitation beam, said optical dispersion means including means for passing predetermined wavelengths of said fluorescence emission spectrum of the particle depending of the position of said particle image with respect to said first surface of said optical dispersion means; and converting means for converting the output from said optical dispersion means to at least one electrical signal representative of at least one of the said predetermined wavelengths of the fluorescence emission spectrum.

3. Apparatus as claimed in claim 2 wherein said converting means comprises a single light sensitive means for converting incident light thereon to electricity and where said detecting means includes focusing means for focusing the output from said optical dispersion means onto said single light sensitive means to produce said electrical signal which varies with time so that said predetermined wavelengths of the fluorescence emission spectrum correspond to respective predetermined points in time of the electrical signal.

4. Apparatus as claimed in claim 2 or 3 wherein said optical dispersion means comprises a wedge filter.

5. Apparatus as claimed in claim 2 or claim 3 wherein said optical dispersion means comprises a prism.

6. Apparatus as claimed in claim 2 or claim 3 wherein said optical dispersion means comprises a grating.

7. Apparatus as claimed in any preceding claim wherein said predetermined wavelengths of said fluorescence emission spectrum constitutes a continuous spectrum.

8. Apparatus as claimed in any preceding claim including means for generating said excitation light beam with a laser.

9. Apparatus as claimed in claim 1 wherein said detecting means sequentially detects said predetermined wavelengths to provide a time varying signal representative of said fluorescence emission spectrum.

10. Apparatus as claimed in claim 9 including means for processing said time varying signal to select therefrom portions corresponding to certain ones of said predetermined wavelengths.

11. Apparatus as claimed in any preceding claim including means for converging said

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excitation light beam into a line coaxial with the path through which said particles pass.

12. Apparatus as claimed in any preceding claim wherein said means for passing the particles

5 includes means for causing said particles to flow single file through said excitation light beam.

13. Apparatus as claimed in claim 2 comprising a light beam source for producing the excitation light beams and wherein the optical dispersion

10 means comprises a wedge filter, there being provided for direction said predetermined wavelength passing through the wedge filter onto the detecting means to provide a time varying signal representative of said fluorescence

15 emission spectrum of said particles.

14. A method for producing the fluorescence emission spectrum of particles comprising the steps of passing said particles through an excitation light beam to effect fluorescence

20 thereof; and detecting with a detecting means predetermined wavelengths of the said fluorescence emission spectrum of each particle, each detected wavelength being a function of the position of said particle with respect to said

25 detecting means as it passes through said excitation light beam.

15. A method as claimed in claim 14 wherein said particles are cells.

16. A method as claimed in claim 14 or claim

30 15 including the step of generating said excitation light beam with a laser.

17. A method as claimed in any of claims 14 to 16 wherein said predetermined wavelength are sequentially detected to provide a time varying signal representative of said fluorescence emission spectrum.

18. A method as claimed in claim 17 including the step of processing said time varying signal to select therefrom portions corresponding to certain ones of said predetermined wavelengths.

19. A method as claimed in claim 14 including projecting an image of each said particle onto a wedge filter so that the particle image moves across the filter as the particle passes through said

40 excitation beam, said wedge filter passing predetermined wavelengths of the said fluorescence emission spectrum of the particle depending on position of said particle image with respect to said wedge filter; and directing said

45 predetermined wavelengths passing through the wedge filter onto a detector to provide a time varying signal representative of said fluorescence emission spectrum of said particles.

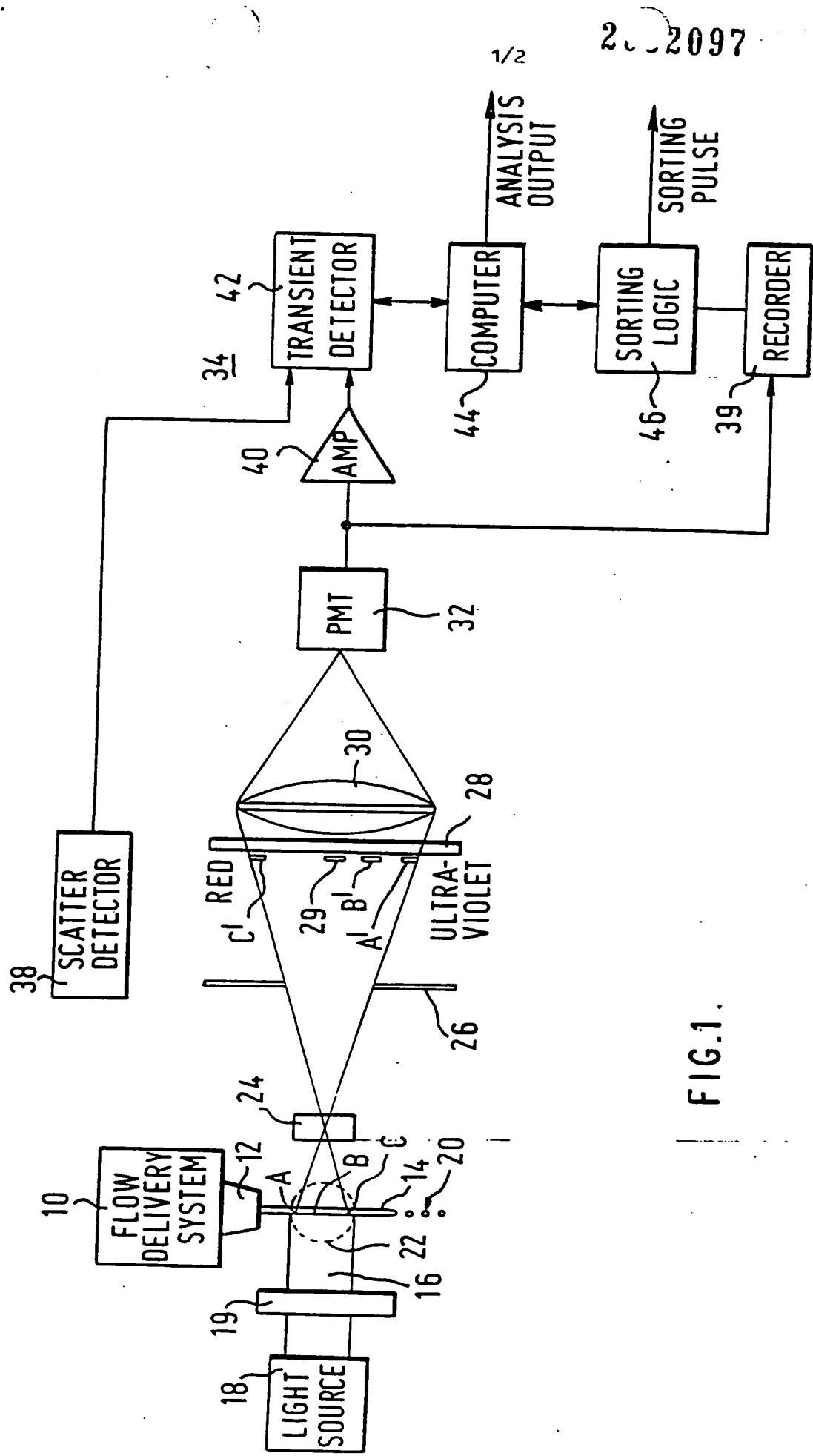
20. Apparatus for producing the fluorescence

55 emission spectrum of particles substantially as hereinbefore described with reference to the accompanying drawings.

21. A method for producing the fluorescence emission spectrum of particles substantially as

60 hereinbefore described with reference to the accompanying drawings.

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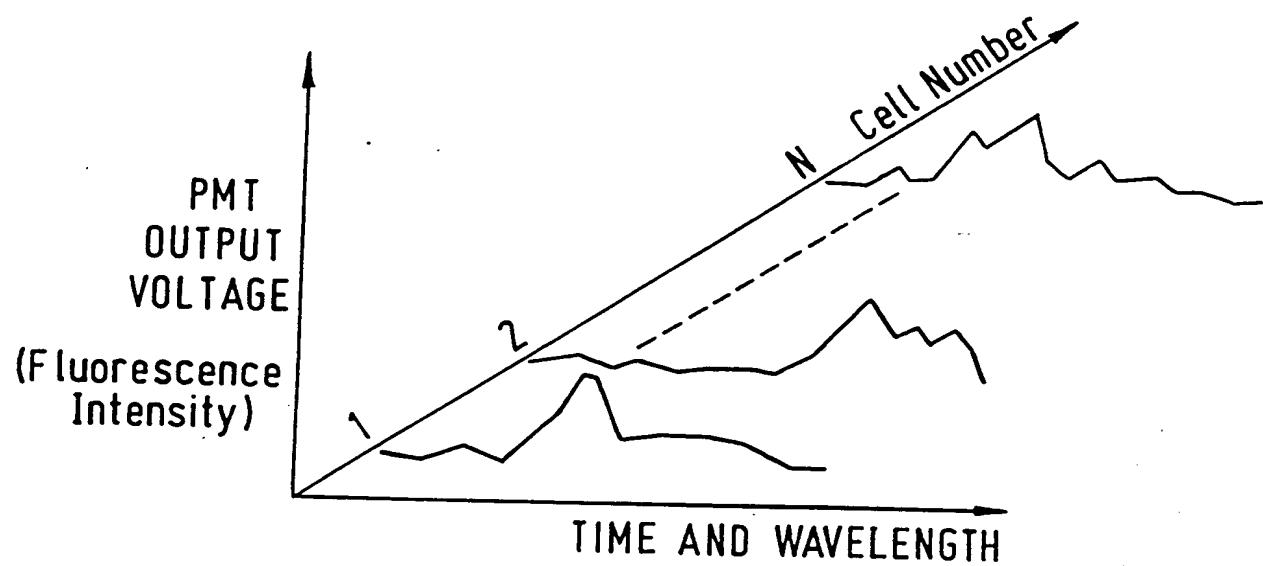


FIG. 2.

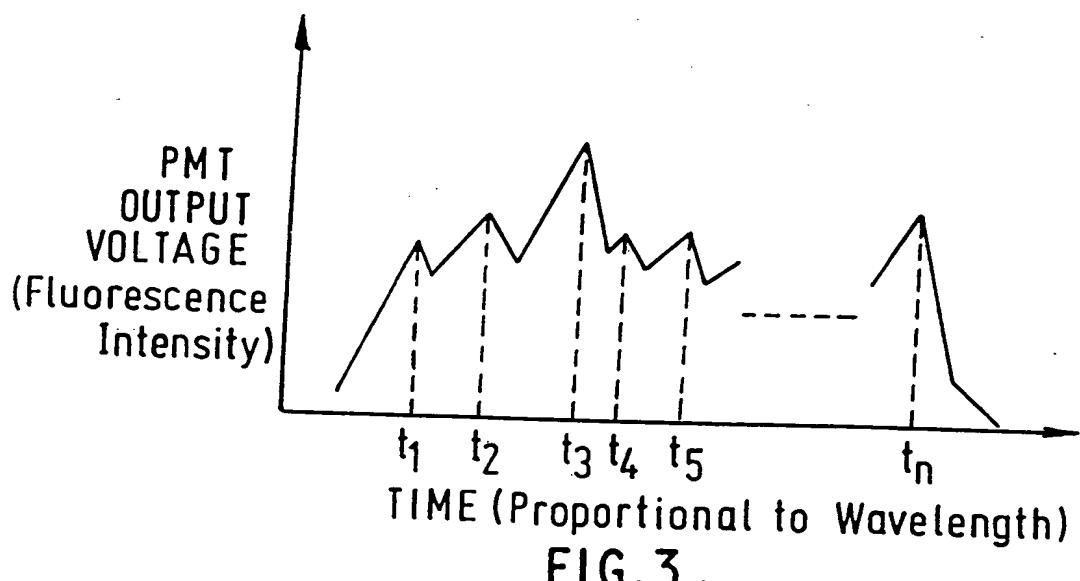


FIG. 3.

## SPECIFICATION

## Apparatus and method of producing the fluorescent emission spectrum of particles

This invention relates to apparatus for and a method of producing the fluorescent emission spectrum of particles.

As is known, the fluorescence spectrum of stained or unstained particles such as human cells can yield valuable information as to the nature of

10 the cell and/or the condition of the cells. For example, the emission spectra of various stained particles within the blood are different. Thus, a count of the different types of white blood cells can be obtained since the emission spectra of 15 these cells are different. Further, it is known the emission spectra of certain stained cancer cells are different from those of normal cells in certain tissues of the body. Again, the differences in emission spectra can be utilized to detect the 20 abnormal presence of pathogenic cells.

It is known to accumulate spectra on individual cells by static single cell analysis or upon a stationary solution containing a number of cells, such as disclosed in U.S. Patents 3 470 373, 25 3 497 690, 3 918 812, and 3 973 129. Further, it is known on a static basis to project a real image of a cell to a predetermined point, there being disposed at a point a rotating or oscillating wedge filter or grating whereby a continuous fluorescence 30 emission spectrum of the static cell can be obtained. However, the accumulation of spectrum information is quite slow due to the static nature of the procedure. The use of a wedge filter in a spectrophotometer is also disclosed in U.S. Patent 35 3 885 879.

Data can be more rapidly accumulated by dynamic single cell analysis in flow microfluorometers where cells are permitted to pass through an excitation light beam to effect 40 fluorescence thereof where, for example, 500—5,000 cells per second can flow through the beam. Such flow microfluorometers are disclosed, for example, in U.S. Patents 3 586 859 3 864 571, and 3 916 197. In some of the latter 45 microfluorometers, at least two filters are employed, each of which passes a different wavelength of the fluorescence emission spectrum of the activated particles. The filter outputs are then respectively focused at at least two different 50 photomultiplier tubes. The outputs of the photomultiplier tubes are then combined for appropriate signal processing.

The resolution of the foregoing flow delivery systems is that only two wavelength portions or 55 lines are detected out of the entire fluorescence emission spectrum of the particles. It is possible to increase this resolution by employing more filters and accordingly more photomultiplier tubes, there being, as indicated above, one photomultiplier tube for each filter. Of course, the resolution can 60 be increased in this manner only to a certain point at which either space limitations or cost limitations occur. Not only is each photomultiplier tube expensive but also there are many

65 applications where space is at a premium.

A further limitation of the above described microfluorometers is that the light supplied to the filters is divided between the filters. Hence, the light available at each filter output for detection is 70 somewhat reduced thus rendering the system somewhat inefficient.

It is accordingly an object of this invention to provide improved apparatus for and a method of producing the fluorescent emission spectrum of particles.

According to one aspect of this invention there is provided apparatus for producing the fluorescence emission spectrum of particles comprising means for passing said particles 80 through an excitation light beam to effect fluorescence thereof; and detecting means for detecting predetermined wavelengths of the said fluorescence emission spectrum of each particle, each detected wavelength being a function of the position of said particle with respect to said detecting means as it passes through the excitation light beam.

According to another aspect of this invention, there is provided a method for producing the 90 fluorescence emission spectrum of particles comprising the steps of passing said particles through an excitation light beam to effect fluorescence thereof; and detecting with a detecting means predetermined wavelengths of 95 the said fluorescence emission spectrum of each particle, each detected wavelength being a function of the position of said particle with respect to said detecting means as it passes through said excitation light beam.

100 This invention can provide a flow microfluorometer apparatus and method whereby a continuous or line fluorescence emission spectrum of a particle can be rapidly obtained.

105 A microfluorometer and method according to this invention may employ a single light detector.

A microfluorometer and method according to the invention can provide the fluorescence emission spectrum of the particle rapidly in a sequential manner.

110 A microfluorometer and method according to this invention can process human cells.

The light may be efficiently utilized at the light detector.

An embodiment of this invention will now be 115 described, by way of example, with reference to the accompanying drawings of which:

Figure 1 is a schematic diagram of flow microfluorometer in accordance with the invention;

120 Figure 2 is an illustrative X—Y oscilloscope display of the spectrum of N cells in accordance with the invention; and

Figure 3 is a graph which illustrates the temporal variation of the spectrum of a cell and 125 how different wavelengths can be obtained at different points in time.

Referring to Figure 1, there is shown a flow delivery system 10 having a nozzle 12 which delivers a particle containing stream 14. The

particles may either be endogenous whereby they fluoresce upon proper excitation thereof or they may be tagged with appropriate substances to effect fluorescence thereof. Further, the particles may

5 comprise cellular matter of either the animal or plant kingdoms. Although there is no intent to be limited to a particular flow rate, typically 500—5,000 cells per second are sequentially delivered from nozzle 12 with a cell separation of

10 0.2—2 cm., the particles transversing a beam 16 emitted from a light source 18 where the cells pass through the beam single file. As indicated at 20, the particle containing fluid tends to break up into drops after passage through the beam. Flow

15 delivery system 10 may correspond exactly to that incorporated in the Fluorescence Activated Cell Sorter (FACS II) manufactured by Becton, Dickinson and Company, Rutherford, New Jersey.

The cross-sectional area of the beam is

20 indicated at 22 in dotted lines, the diameter thereof being typically approximately 1 millimeter. The source 18 may correspond to the laser source used in the above-mentioned Becton, Dickinson FACS II system. If so, the light source is

25 orthogonal to the said detection system at the stream. It should be noted that a 50 micron diameter laser beam is employed in the FACS II system whereas, as mentioned above, the beam width employed in the present embodiment may

30 typically be 1 millimeter. This larger diameter beam can be obtained by simply defocusing the smaller FACS II beam. Of course, laser sources are also known which are capable of generating a 1 millimeter beam. In any event, there is no

35 invention to be limited to a particular width beam nor is there any intent to be limited to a particular type beam or source in that, for example, an ultraviolet source may also be used as source 18.

Further, cylindrical lens or other appropriate

40 one dimensional converging means may be disposed as shown at 19 to converge the beam from source 18 into a line coaxial with the path through the cells travel, the line extending between A and C in Figure 1. By so converging the

45 beam, the intensity thereof is limited to the path travelled by the cells to thereby optimize the utilization thereof.

A particular cell is shown in three different positions A, B and C as it passes through the

50 beam. The cell is, of course, fluorescence activated as it passes through the beam. A lens system 24 and an iris 26 project the image of each cell onto a wedge filter 28, the image of the cell at position A being projected to position A' on filter 28, the

55 image at position B being projected to B' and C to C'. An obscuration bar 29 acts to block the excitation at its transmission region on the wedge filter. In addition to or instead of, a barrier filter can be used to block the excitation. As indicated in

60 Figure 1, the lower end of filter 28 passes radiations at the ultra-violet end of the spectrum, the upper end passes red wavelengths while position B' may correspond to blue wavelengths. Hence, although the entire fluorescence emission spectrum is projected onto A' when the cell is at

A, only a short wavelength portion of the spectrum (ultraviolet, for example) is passed by the filter. At B', a longer wavelength portion is passed (blue, for example) while at C' the longest wavelength

70 portion of the spectrum is passed (red, for example).

The radiations transmitted from filter 28 are sequentially focused by a collecting lens 30 onto a photomultiplier tube 32. That is, when the cell

75 image is at A', the shorter wavelength portion of the fluorescence emission spectrum is focused onto the photomultiplier tube. As the cell passes through the beam 16, successively longer wavelength portions of the emission spectrum will

80 be focused at tube 32 until the cell image reaches C' at which time the longest wavelength of interest will be directed to the tube. Hence, the output signal from photomultiplier tube 32 which is applied to a signal processor 34 and a recorder

85 39 is a time varying signal, the time axis thereof being proportional to wavelength and the voltage or current output being proportional to the emission intensity at a particular wavelength. It can thus be seen from the foregoing that there is provided a

90 unique method for scanning the entire fluorescence emission spectrum of particles such as cells, this being effected by projecting the moving image of the particle as it passes through an excitation beam onto a wedge filter which

95 thereby sequentially transmits increasingly longer (shorter) wavelength portions of the spectrum to a detection device including lens 30 and tube 32. In lieu of wedge filter 28, other optical dispersion means may be used such as a prism or grating

100 where suitable geometry would be employed and the principle would be the same. Also, in lieu of lens 30 and tube 32, a linear light sensitive, diode array may be placed in the position of lens 30 adjacent filter 28 whereby the lowest diode would

105 be responsive to the ultraviolet portion (for example) of the spectrum and the highest, the red portion (for example) thereof. In this case, the outputs from the diodes would still occur sequentially; however, they would be in parallel

110 and suitable for processing by appropriate circuitry. It should also be noted that although the wedge filter 28 has been shown with the red end thereof at the top and the ultraviolet end at the bottom, these ends could be reversed so that red occurs at the bottom and ultraviolet at the top.

115 The spectrum as generated above is the so-called uncorrected spectrum and may be corrected by correcting for the system transmission function either electronically or by the use of spatial filters as is known in this art.

The output signal from photomultiplier tube 32 is applied to a signal processor generally indicated at 34 the operation of which may be initiated by the output of a scatter detector 38. Scatter detector 38 is responsive to light scattered from each cell when it reaches point A. That is, prior to the cell

120 reaching point A, it does not fluoresce. Upon reaching point A, the fluorescence is activated by the laser beam and, in addition to the rays projected through lens system 24 and iris 26,

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there are rays which impinge upon detector 38. Detector 38 includes a photodetecting element and may correspond exactly to the scatter detector used in the above-mentioned FACS II system. Photomultiplier tube 32 may also correspond to the tube used in the FACS II system as may lens system 24 and iris 26. Scatter detector 38 thus signals to processor 34 the presence of a particle in beam 16. The output from photomultiplier tube 32 may also be applied to a recorder or display 39 such as an oscilloscope where the fluorescence spectrum may be visually inspected and photographed if desired. For example, X-Y oscilloscope may be employed to isometrically display each pulse (or cell) as shown in Figure 2.

Signal processor 34 effects the extraction of more information. Thus, it permits analysis of the entire output waveform from tube 32 and computer analysis of the entire cell population. Thus, the output waveform is applied to a transient waveform detector 42 via an amplifier 40 and then fed to a computer 44. Detector 42 and computer 44 are commercially available and the storing logic may be the same as that used in the FACS II system. With appropriate *a priori* knowledge, it is possible to quantitate those compounds which fluoresce in the particle. Wavelength variation in spectra can be obtained at different points in time as indicated in Figure 3. Each  $t_i$  corresponds to a particular fluorescence wavelength, and the value of  $n$  is determined by the overall resolution of the chemical (tagging) optical and electronics system. An appropriate data reduction scheme may be used to obtain descriptors of the spectrum which then may be used as a means of identifying similar cell types.

It is possible to physically isolate cell types according to the values of fluorescence at a particular wavelength or by use of the descriptors. Since the grouping of the cells is dependent upon its identification, it suffices only to find the appropriate means of analysis and to sort in accordance with sorting logic 46 on the basis of this analysis, as is possible on the cell sorter-type of instrument. Care is required to consider uniqueness for the descriptors and the time available to perform the sorting operation.

It is apparent that many schemes exist to analyze and sort cells on the basis of their fluorescence spectra as measured in the present invention. For example, one can measure the fluorescence depolarization spectrum of single particles by appropriately splitting the emission beam with polarizing filters and using two of the units of the present invention. Both the parallel and perpendicular fluorescence quantities are available for calculation using the approach of processor 34 of Figure 1. Many other examples can be implemented by using alternate schemes with the described embodiment of the present invention and appropriated electronics.

## CLAIMS

## 1. Apparatus for producing the fluorescence

65 emission spectrum of particles comprising means for passing said particles through an excitation light beam to effect fluorescence thereof; and detecting means for detecting predetermined wavelengths of the said fluorescence emission spectrum of each particle, each detected wavelength being a function of the position of said particle with respect to said detecting means as it passes through the excitation light beam.

70 2. Apparatus as claimed in claim 1 wherein said detecting means includes optical dispersion means having at least a first surface; means for projecting and image of each said particle onto said optical dispersion means so that the particle image moves across said first surface as the particle passes through said excitation beam, said optical dispersion means including means for passing predetermined wavelengths of said fluorescence emission spectrum of the particle depending on the position of said particle image with respect to said first surface of said optical dispersion means; and converting means for converting the output from said optical dispersion means to at least one electrical signal representative of at least one of the predetermined wavelengths of the fluorescence emission spectrum.

75 3. Apparatus as claimed in claim 2 wherein said converting means comprises a single light sensitive means for converting incident light thereon to electricity and where said detecting means includes focusing means for focusing the output from said optical dispersion means onto said single light sensitive means to produce said electrical signal which varies with time so that said predetermined wavelengths of the fluorescence emission spectrum correspond to respective predetermined points in time of the electrical signal.

80 4. Apparatus as claimed in claim 2 or 3 wherein said optical dispersion means comprises a wedge filter.

85 5. Apparatus as claimed in claim 2 or claim 3 wherein said optical dispersion means comprises a prism.

90 6. Apparatus as claimed in claim 2 or claim 3 wherein said optical dispersion means comprises a grating.

95 7. Apparatus as claimed in any preceding claim wherein said predetermined wavelengths of said fluorescence emission spectrum constitutes a continuous spectrum.

100 8. Apparatus as claimed in any preceding claim including means for generating said excitation light beam with a laser.

105 9. Apparatus as claimed in claim 1 wherein said detecting means sequentially detects said predetermined wavelengths to provide a time varying signal representative of said fluorescence emission spectrum.

110 10. Apparatus as claimed in claim 9 including means for processing said time varying signal to select therefrom portions corresponding to certain ones of said predetermined wavelengths.

115 11. Apparatus as claimed in any preceding claim including means for converging said

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excitation light beam into a line coaxial with the path through which said particles pass.

12. Apparatus as claimed in any preceding claim wherein said means for passing the particles

5 includes means for causing said particles to flow single file through said excitation light beam.

13. Apparatus as claimed in claim 2 comprising a light beam source for producing the excitation light beams and wherein the optical dispersion

10 means comprises a wedge filter, there being provided for direction said predetermined wavelength passing through the wedge filter onto the detecting means to provide a time varying signal representative of said fluorescence

15 emission spectrum of said particles.

14. A method for producing the fluorescence emission spectrum of particles comprising the steps of passing said particles through an excitation light beam to effect fluorescence

20 thereof; and detecting with a detecting means predetermined wavelengths of the said fluorescence emission spectrum of each particle, each detected wavelength being a function of the position of said particle with respect to said

25 detecting means as it passes through said excitation light beam.

15. A method as claimed in claim 14 wherein said particles are cells.

16. A method as claimed in claim 14 or claim

30 15 including the step of generating said excitation light beam with a laser.

17. A method as claimed in any of claims 14 to 16 wherein said predetermined wavelength are sequentially detected to provide a time varying signal representative of said fluorescence emission spectrum.

18. A method as claimed in claim 17 including the step of processing said time varying signal to select therefrom portions corresponding to certain

40 ones of said predetermined wavelengths.

19. A method as claimed in claim 14 including projecting an image of each said particle onto a wedge filter so that the particle image moves across the filter as the particle passes through said

45 excitation beam, said wedge filter passing predetermined wavelengths of the said fluorescence emission spectrum of the particle depending on position of said particle image with respect to said wedge filter; and directing said

50 predetermined wavelengths passing through the wedge filter onto a detector to provide a time varying signal representative of said fluorescence emission spectrum of said particles.

20. Apparatus for producing the fluorescence

55 emission spectrum of particles substantially as hereinbefore described with reference to the accompanying drawings.

21. A method for producing the fluorescence emission spectrum of particles substantially as

60 hereinbefore described with reference to the accompanying drawings.